

## **CLAIMS**

1. A method of extracting a polypeptide of interest from a fermentation broth comprising:
  - 5    i) adjusting the pH close to pI of the polypeptide of interest;
  - ii) adding a non-ionic surfactant with a hydrophile-lipophile balance (HLB) of 12 or lower;
  - iii) cooling the mixture for solubilization and incubating at above cloud point for extraction;
- 10   iv) phase separating at below cloud point to obtain liquid-liquid-solid fractions; and
- v) recovering the surfactant-rich top phase containing the polypeptide of interest.
  
2. The method according to claim 1, wherein the polypeptide of interest is an enzyme or a peptide.
  

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3. The method according to claim 2, wherein the enzyme is selected from the group consisting of a protease, an amylase, a cellulase, a lipase, an oxidoreductase, and a carbohydrolase.
  
- 20   4. The method according to claim 2, wherein the peptide contains from 5 to 100 amino acids.
  
5. The method according to claim 1, wherein the pH is adjusted to be in the range of (pH-pI) of -3 to +1.
  

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6. The method according to claim 5, wherein the pH is adjusted to be in the range of (pH-pI) of -2 to -1.
  
7. The method according to claim 1, wherein the pH is adjusted to a value of 1.3 units  
30   below isoelectric point.

8. The method according to claim 1, wherein the hydrophile-lipophile balance (HLB) is in the range of from 7 to 12.
9. The method according to claim 1, wherein the non-ionic surfactant is selected from  
5 the group consisting of an alcohol ethoxylate, a fatty acid ester, a polyether alcohol and an amine oxide.
10. The method according to claim 9, wherein the non-ionic surfactant is a linear fatty alcohol ethoxylate.
11. The method according to claim 1, wherein the non-ionic surfactant is added in an amount of 5 to 25% (w/w).
12. The method according to claim 1, wherein the mixture is cooled to 3-10°C for  
15 solubilization; preferably to 4-6°C for solubilization; in particular to 5°C for solubilization.
13. The method according to claim 1, wherein the mixture is incubated at 2-10°C above cloud point for extraction; preferably at 3-9°C above cloud point for extraction.
- 20 14. The method according to claim 1, wherein the phase separating is done at 2-15°C below cloud point for extraction; preferably at 3-11°C below cloud point for extraction.
15. The method according to claim 1 additionally comprising a step vi):  
vi) concentrating the extracted mixture to a paste form.
- 25 16. The method according to claim 15, wherein in step vi) the extracted mixture is concentrated to a paste form after adjusting the pH to neutral.
17. The method according to claim 1, wherein in step i) the fermentation broth is diluted  
30 (0 to 100%) for viscosity reduction before adjusting the pH close to pl of the polypeptide of interest.

18. The method according to claim 1, wherein the fermentation broth to be adjusted in step i) is a clarified or a whole fermentation broth.